Name _____ Date _____

Partner(s) _____

OSMOSIS AND DIFFUSION LAB

Cells must move materials through membranes and throughout cytoplasm in order to maintain homeostasis. The movement is regulated because cellular membranes, including the plasma and organelle membranes, are selectively permeable. Membranes are phospholipid bilayers containing embedded proteins. The phospholipid fatty acids limit the movement of water because of their hydrophobic characteristics.

The cellular environment is aqueous, meaning that the solutes (e.g., salts, organic molecules) dissolve in water, which is the solvent. Water may pass freely through the membrane by osmosis or through specialized protein channels called aquaporins. Most ions move through protein channels, while larger molecules, such as carbohydrates, are carried by transport proteins.

The simplest form of movement is diffusion, in which solutes move from an area of high concentration to an area of low concentration; diffusion is directly related to molecular kinetic energy. Diffusion does not require energy input. The movement of a solute from an area of low concentration to an area of high concentration requires energy input in the form of ATP and protein carriers called pumps.

Water moves through membranes by diffusion; this process is called osmosis. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential (high water concentration) and low solute concentration to areas of low potential (low water concentration) and high solute concentration. In walled cells, osmosis is affected not only by the solute concentration but also by the resistance to water movement in the cell by the cell wall. This resistance is called turgor pressure.

The terms hypertonic, hypotonic, and isotonic are used to describe solutions separated by selectively permeable membranes. A hypertonic solution has a higher solute concentration and a lower water potential as compared to the other solution; therefore, water will move into the hypertonic solution through the membrane. A hypotonic solution has a lower solute concentration and a higher water potential than the solution on the other side of the membrane; water will move down its concentration gradient into the other solution. Isotonic solutions have equal water potential.

The movement of solutes and water across cellular membranes is an overarching concept. Cells must maintain their internal environments and control solute movement. These concepts can be illustrated using living cells. The purpose of this lab is to demonstrate the properties of osmosis and diffusion using both plant and animal cells.

Terms

Diffusion: The random movement of molecules or particles, resulting in the net movement of a substance from a region of high concentration to a region of low concentration.

Osmosis: The diffusion of water across a semi-permeable membrane.

Plasmolysis: A phenomenon in walled cells in which the cytoplasm shrivels and the plasma membrane pulls away from the cell wall; occurs when a cell loses water to a hypertonic environment

Solute: A substance that is dissolved in a solution.

Solution: A homogeneous, liquid mixture of two or more substances.

Semi-permeable membrane: A membrane that allows some molecules, but not others, to pass through it.

You will complete the following lab assignment in class. You will work in groups of four. Work together to answer the following question, but make sure you write your own answers. All members of each group must submit their own lab packet. It will be collected at the end of your lab period and is worth 40 points.

PART 1: Re-familiarize yourself with the microscope
What is the magnification of the:

Scanning Objective _____

Low Power Objective _____

High Power Objective _____

Are these magnification equal to the total magnification of the microscope? [YES / NO]

There are two types of focus. What are they?

1. _____
2. _____

When do you use each type of focus (with which objective lens?)

1. _____
2. _____

You are looking at a prepared slide through a microscope. What happens when you move the slide to the left? Which direction does your sample move?

PART 2: Preparing NaCl solutions

Many experiments involving chemicals call for their use in solution form. That is, two or more substances are mixed together in known quantities. This may involve weighing a precise amount of dry material or measuring a precise amount of liquid. Preparing solutions accurately will improve an experiment's safety and chances for success.

For today's lab, we will prepare a solution using a method called "percentage by weight".

The formula for weight percent (w/v) is: [Mass of solute (g) / Volume of solution (ml)] x 100

Example: A 10% NaCl solution has ten grams of sodium chloride (NaCl) dissolved in 100 ml of solution.

Question: How many grams of NaCl would you dissolve in 100 ml of water if you wanted to make a 40% NaCl solution?

_____

For this lab, you will need to prepare a 20% NaCl solution, and a 30% NaCl solution. You will not need very much, however, so you don't need to prepare a large quantity. Partner with another group; you should each prepare one of the solutions.

20% NaCl Procedure:

Weigh 2 g of sodium chloride. Pour it into a graduated cylinder containing about 8 ml of water. Once the sodium chloride has dissolved completely (swirl the flask gently if necessary), add water to bring the volume up to the final 10 ml. Caution: Do not simply measure 10 ml of water and add 2 g of sodium chloride. This will introduce error because adding the solid will change the final volume of the solution and throw off the final percentage. Transfer solution to a beaker for use in the experiment. Be sure to label the beaker with the correct concentration.

30% NaCl Procedure:

Weigh 3 g of sodium chloride. Pour it into a graduated cylinder containing about 8 ml of water. Once the sodium chloride has dissolved completely (swirl the flask gently if necessary), add water to bring the volume up to the final 10 ml. Caution: Do not simply measure 10 ml of water and add 2 g of sodium chloride. This will introduce error because adding the solid will change the final volume of the solution and throw off the final percentage. Transfer solution to a beaker for use in the experiment. Be sure to label the beaker with the correct concentration.

IF SALT DOES NOT DISSOLVE INTO 20% OR 30% SOLUTIONS, INFORM YOUR INSTRUCTOR BEFORE PROCEDING TO THE NEXT STEPS. ADJUSTMENTS TO THE PROTOCOL MAY BE NECESSARY.

PART 3: Osmosis in red onion cells

1. Obtain 1 blank microscope slide.
2. Prepare a single layer of red onion cells:
    - We need a thin layer of cells of the red part of the onion. It is not possible to directly cut a single cell layer, so we need to use the "peeling method" to obtain a single layer of cells. Obtain a small piece of onion about (1cm x 1cm). The onion layer is about 2mm thick.
    - With the red side of the onion facing you, cut beneath the red layer, about half way into the onion. This cut does not have to be very thin. There will be about 1mm of onion between the knife and the red pigmented layer.
    - Press the onion firmly against the blade with your thumb.
    - Now tear off or peel away the red part of the onion. The red layer will become thin. Some red pigment may be released from broken cells.
    - Cut away and discard the thick part of the onion (the place where the initial cut was placed).
3. Place your thin section of red onion cells on the center of the microscope slide. Add a few drops of DI water, then place a coverslip onto the slide, taking care to avoid air bubbles. Remember to place the cover slip at a 45 degree angle and slowly drop the cover slip onto your sample.

4. Are red onion cells plant cells or animal cells?

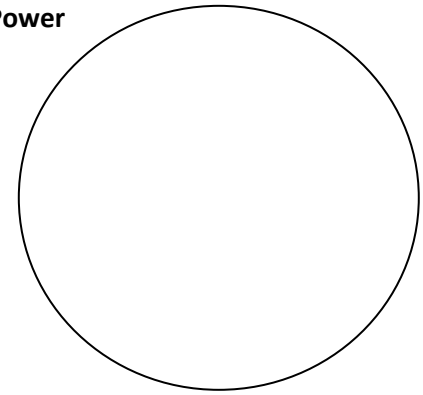
5. Make a prediction. What do you think the red onion cells will look like under this condition?

6. Image your cells. Use the SCANNING objective to focus. Only consider those cells which have red pigment. White cells are those which have had their membrane disrupted and have lost the red pigment.

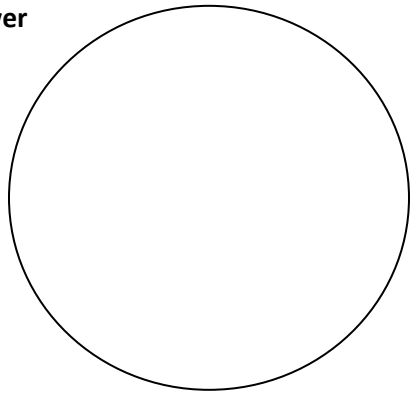
   Switch to LOW POWER and focus. It will likely be possible to focus in multiple panes of your sample given its thickness. Find the pane where you have the most cells in focus. Sketch your cells at low power below. Make sure you draw your cells to scale.

   Once you think you have located a cell with red pigment, switch to HIGH POWER and refocus. (Remember, do NOT use the coarse adjustment knob at this point). Sketch your cell at high power below. Label the cell wall, cytoplasm, and red pigment on your high power image. Can you identify any other features? A nucleus? Make sure to draw your cells to scale.

Low Power



High Power



Low Power Total Magnification _____

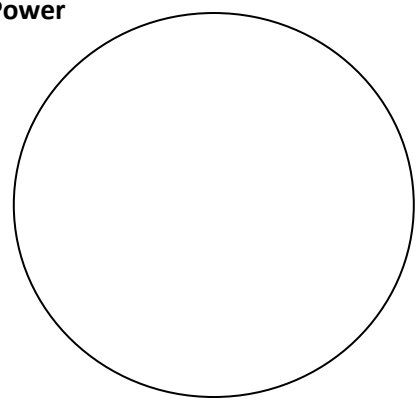
High Power Total Magnification _____

(Remember, Total Magnification = Ocular Magnification x Objective Magnification)

7. Next, we will alter the chemical environment of the cells by adding several drops of the 30% NaCl solution you prepared to one side of the cover slip (left or right). There is no need to remove the cover slip to add the solution; the 30% NaCl solution should flow underneath the coverslip on its own. Place a scratch-resistant lens cloth on the opposite side of the cover slip to help pull out the DI water.
8. Make a prediction. What do you think will happen to the cells?

9. Watch what happens to the red pigment inside the cells. After several minutes, draw what you see. You will only draw your cells at high power. Label the cell wall, cytoplasm, and red pigment on your high power image. Can you identify any other features? A nucleus? Make sure to draw your cells to scale.

High Power



High Power Total Magnification _____

(Remember, Total Magnification = Ocular Magnification x Objective Magnification)

10. Clean your slide and coverslip with warm soapy water and pat dry with a scratch-resistant lens cloth. Return any other materials to the front of the classroom.
11. Answer the post-lab questions about this section of the lab.

PART 3: Osmosis in epithelial cheek cells

1. Obtain 2 blank microscope slides.
2. On one slide, place one drop of DI water and one drop of methylene blue (a cellular stain) in the center of the slide. Caution: methylene blue will stain clothes and skin.
3. One the other slide, place one drop of 20% NaCl solution and one drop of methylene blue in the center of the slide.
4. Take a toothpick and gently scrape the inside of your cheek with the flat side of a toothpick to remove cheek epithelial cells. Stir the end of the toothpick into the stain/DI water solution, then throw the toothpick away.
5. Repeat this process for the stain/20% NaCl solution slide.
6. Carefully, place a coverslip onto the slide, taking care to avoid air bubbles. Remember to place the cover slip at a 45 degree angle and slowly drop the cover slip onto your sample.
7. Are epithelial cheek cells plant cells or animal cells?

8. Make a prediction. How do you think the cells will look under each of these conditions?

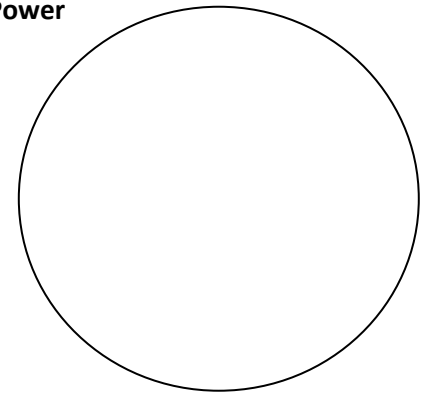
9. Image your cells in stain/DI water first.

   Use the SCANNING objective to focus. You probably will not see the cells at this power.

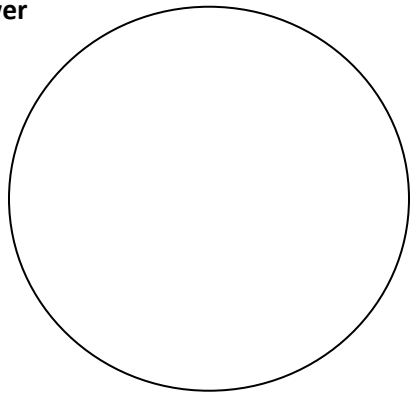
Switch to LOW POWER. Cells should be visible, but they will be small and look like nearly clear purplish blobs. If you are looking at something very dark purple, it is probably not a cell. Sketch your cells at low power below. Make sure you draw your cells to scale.

Once you think you have located a cell, switch to HIGH POWER and refocus. (Remember, do NOT use the coarse adjustment knob at this point). Sketch your cell at high power below. Label the nucleus, cytoplasm, and cell membrane on your high power image. Make sure to draw your cells to scale.

Low Power



High Power



Low Power Total Magnification _____ **High Power Total Magnification _____**

(Remember, Total Magnification = Ocular Magnification x Objective Magnification)

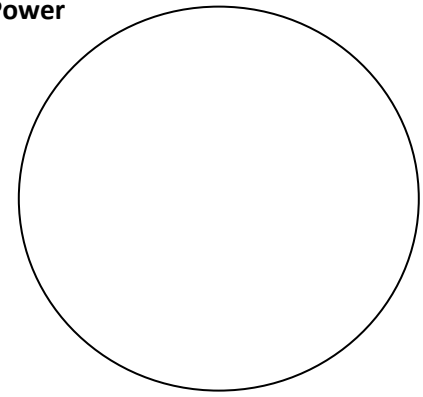
10. Clean your slides using warm soapy water and dry with a scratch-resistant cloth.
11. Next, image your cells in stain/20% NaCl solution.

Use the SCANNING objective to focus. You probably will not see the cells at this power.

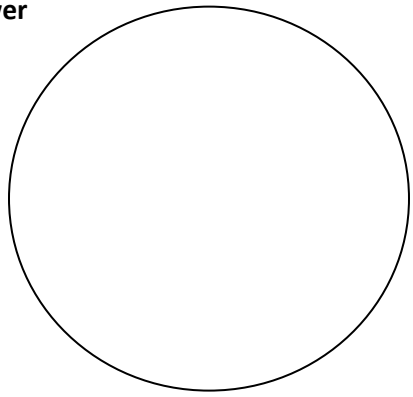
Switch to LOW POWER. Cells should be visible, but they will be small and look like nearly clear purplish blobs. If you are looking at something very dark purple, it is probably not a cell. Sketch your cells at low power below. Make sure you draw your cells to scale.

Once you think you have located a cell, switch to HIGH POWER and refocus. (Remember, do NOT use the coarse adjustment knob at this point). Sketch your cell at high power below. Label the nucleus, cytoplasm, and cell membrane on your high power image. Make sure to draw your cells to scale.

Low Power



High Power



Low Power Total Magnification _____ **High Power Total Magnification _____**

(Remember, Total Magnification = Ocular Magnification x Objective Magnification)

12. Clean your slides and cover slips using warm soapy water, then pat dry with a scratch-resistant cloth.
13. Answer the post-lab questions about this section of the lab.

Post Lab Questions. These questions are due at the end of your lab period. You should work with your group to answer the questions but you should write your own response.

1. When observing the red onion cells in DI water, how did the cells look? How would you describe the environment that the cells were in? Hypotonic, isotonic, or hypertonic? How do you know?

2. What happened to the red onion cells when they became suspended in the 30% NaCl solution? How would you describe the environment that the cells were in? Hypotonic, isotonic, or hypertonic? How do you know?

3. Describe plasmolysis. Did you visualize this today? Was it present in all cells within your field of view? Why or why not? Is this condition reversible?

4. What is the normal tonicity state for a plant cell?

5. Why is the addition of methylene blue necessary when visualizing epithelial cheek cells?

6. When observing the epithelial cheek cells in DI water, how did the cells look? How would you describe the environment that the cells were in? Hypotonic, isotonic, or hypertonic? How do you know?

7. What happened to the epithelial cheek cells when they became suspended in the 20% NaCl solution? How would you describe the environment that the cells were in? Hypotonic, isotonic, or hypertonic? How do you know?

8. What is the normal tonicity state for an animal cell?

9. Can plasmolysis occur in animal cells? Why or why not?

10. Is the cheek cell a eukaryote or prokaryote? How do you know?

11. Keeping in mind that the mouth is the first site of chemical digestion in a human. Your saliva starts the process of breaking down the food you eat. Keeping this in mind, what organelle do you think would be numerous inside the cells of your mouth?